

nervi trigemini where all the fibres lay immediately medial and dorsomedial to this nucleus¹⁶.

Zusammenfassung. Nach einseitigen Läsionen im Rattenhirn an der Grenze zwischen Pons und Mesencephalon, konnte auf Grund von anterograden und retrograden

neuronalen Veränderungen gezeigt werden, dass mindestens $\frac{2}{3}$ der noradrenergen Endigungen im Telencephalon und Diencephalon hauptsächlich zu den ungekreuzten, von Zellkörpern aus Pons und Medulla oblongata stammenden, Axonen gehören.

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The Effect of some Neurohormones on the Heart Rate of Spiders

The authors of the present paper studied the effect of acetylcholine and adrenalin on the heart rate of the spider *Tegenaria atrica* C. L. Koch, by the method used by MIKULSKA and KOKOCIŃSKI¹ and using electroencephalographic recording.

Isolated weighed abdomens were immobilized on the stage of a stereoscopic microscope, after which two steel needle electrodes of 5–10 μ diameter, were introduced hypodermally at two symmetrical points with the help of a micromanipulator. The electrodes were connected to a Kaiser electroencephalograph and the actional potentials of the heart were recorded at a time constant of 0.6 or 1.0 sec, the instrument being calibrated at 50 μ V/3.5 mm and the high frequency filter on.

The intracardiac injections of adrenalin and acetylcholine were made using an Agla microsyringe connected to glass needles controlled by means of a micromanipulator. The required concentrations of the neurohormones

were obtained by diluting them in Jager physiological solution. Test injections did not produce any change in the action of the heart.

The results obtained so far are as follows: injections of adrenalin in a concentration of 10^{-4} caused a threshold effect in the form of a slight acceleration of the heart rate, but without any definite change in the amplitude of the curve. The threshold amount of adrenalin per 1 mg of preparation giving a positively chronotropic effect was $1.1 \cdot 10^{-6}$ mg. Dosed $5.2 \cdot 10^{-6}$ mg per 1 mg, adrenalin gave an acute effect characterized by a rapid decrease of the amplitude and an acceleration of the heart rate. The return to normal was comparatively slow (Figure 1). Further increase of the adrenalin dose led to a levelling of the amplitude and to a stop in the action of the heart.

The injections of acetylcholine gave a negatively chronotropic effect. Already in a concentration of

¹ I. MIKULSKA and W. KOKOCIŃSKI, Bull. Acad. Pol. Sci., Warszawa, in press (1965).

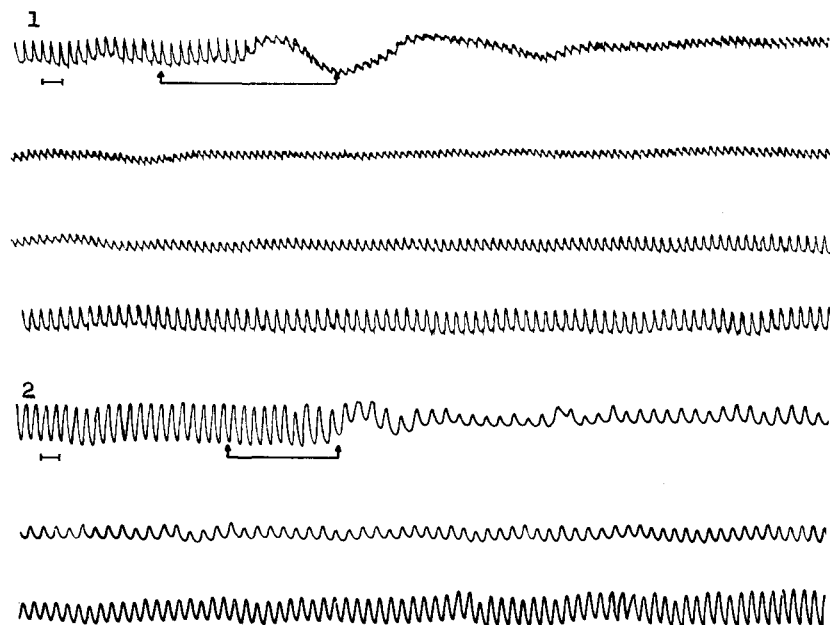


Fig. 1. Electrogram of heart in isolated abdomen. Injection of adrenalin $5.2 \cdot 10^{-6}$ mg of weight of abdomen. $\blacktriangle \rightarrow \blacktriangle$ = injection; $| \text{---} |$ = sec.

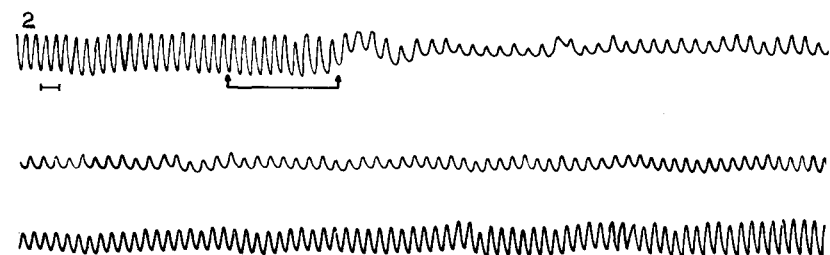


Fig. 2. Electrogram of heart in isolated abdomen. Injection of acetylcholine $2.6 \cdot 10^{-6}$ mg of weight of abdomen $\blacktriangle \rightarrow \blacktriangle$ = injection; $| \text{---} |$ = sec.

$1.7 \cdot 10^{-6}$ mg/mg of preparation, acetylcholine caused a slight slowing down of the heart action, but no inotropic effect was observed. An injection of $2.6 \cdot 10^{-6}$ mg/mg of weight was followed by a pronounced slowing down of the heart rate and a decrease of the amplitude (Figure 2). The return to normal, however, was quicker than after adrenalin. Overdosing resulted, as with adrenalin, in stopping the heart action.

Any comparison of the above results with those for other invertebrates is difficult because of the difference in the methods used. Certain analogies, however, may be found between the effect of acetylcholine in spiders and that in molluscs² and lower crustaceans³. The effect of adrenalin, on the other hand, is similar with regard to the chronotropic effect, but the reverse in the inotropic effect.

Résumé. Les auteurs ont étudié l'effet de l'adrénaline et de l'acétylcholine sur le rythme du cœur chez l'arai-

gnée *Tegenaria atrica* C. L. Koch. L'enregistrement s'est fait au moyen de l'électroencephalographe. L'injection de l'adrénaline provoquait l'accélération du rythme du cœur, l'acétylcholine – son ralentissement.

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Department of Neurophysiology and Comparative Physiology and Department of Systematic Zoology, Copernicus University, Torun (Poland), June 28, 1965.

² C. L. PROSSER, *Comparative Animal Physiology* (Philadelphia 1952).

³ T. WATERMANN, *The Physiology of Crustacea* (New York 1960).

Autotrophic Incorporation of $C^{14}O_2$ in *Cuscuta australis* in Relation to its Parasitism

Introduction. Although *Cuscuta* is traditionally regarded as a classic example of parasite plant, unable to grow autotrophically, several reports in the literature of less recent years suggest the possibility of dodder photosynthesis. Some of these concern the presence of chlorophyll¹, others the light-induced oxygen evolution². More recent references concern the possibility of *Cuscuta* growing and synthesizing starch in vitro culture without an external sugar supply^{3,4} and this is difficult to explain from a point of view of a total heterotrophy.

Recently⁵ it has been found that $C^{14}O_2$ fixation in two species of *Cuscuta* is a photosynthetic process and virtually all the radioactivity is present in the sucrose zone. On the contrary, CIFERRI^{6,7} reported that *C. epythymum* could really carry out only a 'heterotrophic fixation' through the carboxylation of phosphoenolpyruvic acid.

In order to reinvestigate the whole problem of the photosynthetic activity of *Cuscuta*, we have examined both the pigment composition of this plant^{8,9} and the light-driven $C^{14}O_2$ incorporation into organic compounds.

This report deals with this latter topic.

Methods. Seedlings of *C. australis* were utilized, which were grown for 6 days in continuous light at $25 \pm 1^\circ C$ and stems of the same dodder detached from the host (*Medicago sativa*) at various stages of growth. Before the exposure to $C^{14}O_2$, the seedlings were kept 12 h in the dark and 15 min in the light, the stems were kept on moistened filter paper for 6 h in the dark and 15 min in the light. Then seedlings or stems were placed in plexiglass containers (250 ml) and exposed to an atmosphere containing $C^{14}O_2$ ($30 \mu C Na_2C^{14}O_3$, sp. act. $1.16 mC/mM$, together with sufficient carrier to give a final atmosphere of 2% CO_2 within the box). After 1 h CO_2 was evacuated from the chambers and the plants homogenized twice more in boiling 80% ethanol. The combined alcoholic extracts were evaporated to dryness under reduced pressure at $40^\circ C$. The residue was extracted with ethyl ether and ethanol-ether (3:1), dissolved in distilled water, and again evaporated. Then it was dissolved in glycine buffer

pH 8.5 and incubated for 2 h at $37^\circ C$ with alkaline phosphatase and Mg^{++} ($5 \cdot 10^{-4} M$).

The water-soluble material was fractionated with Dowex resins in 1.6 cm columns. The basic fraction (amino acids) was eluted from Dowex 50W $\cdot 8$ (100–200 mesh) (H^+) with $0.25 N NaOH$, and the acidic fraction (organic acids) from Dowex $1 \cdot 10$ (200–400 mesh) (formate form) with $8 N$ formic acid.

The residue from alcohol extraction was hydrolysed for 3 h in 10 ml of $1 N H_2SO_4$ in boiling water. It was neutralized with $BaOH$, filtered, and the excess of $BaOH$ precipitated. The same methods were applied to the hydrolysed as to the water-soluble material.

A sample of all the fractions was evaporated to dryness on a planchette and analysed for radioactivity on a windowless gas-flow counter.

Aliquots of the neutral fractions of the ethanol soluble extract were applied to Whatman No. 1 filter paper and chromatographed using the upper layer of a mixture of ethylacetate-acetic acid-water (3:1:3)¹⁰. Known components in concentrations of 25–50 μg were simultaneously chromatographed. Sheets of X-ray film, $40 \cdot 25$ cm, were exposed to the chromatographs and subsequently developed in the usual manner. Spots on the radiochromatographs were identified by means of specific spray reagents, comparison of Rf values with those of standards and cochromatography.

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⁴ F. BERTOSSI, *Atti Ist. Bot. Lab. Critt. Pavia* 14, 174 (1956).

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⁷ O. CIFERRI, F. SALA, and G. POMA, *Riv. Pat. Veg.*, 4, 521 (1964).

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